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REVIEW / PRACA POGLĄDOWA

Aleksandra Kołota, Joanna Gromadzka-Ostrowska

OXIDO-REDUCTIVE STRESS AND ALCOHOLIC LIVER DISEASE

STRES OKSYDOREDUKCYJNY A ALKOHOLOWA CHOROBA WĄTROBY

Chair of Nutritional Physiology, Department of Dietetics, Faculty of Human Nutrition and Consumer Sciences,
Warsaw University of Life Sciences
Head of the Chair of Nutritional Physiology: prof. dr hab. Joanna Gromadzka-Ostrowska

S u m m a r y

Due to availability and relatively low price, alcohol is currently the most widely used intoxicant. Consuming excessive amounts of alcohol is associated with multiple health consequences, which include pathological changes in the liver leading to development of alcoholic liver disease. Responsible for the formation of the mentioned changes are among others: metabolic disorders, immune reactions and formation of cytokines, hypoxia of hepatocytes in the perivenular area of hepatic lobule and oxido-reductive stress. The aim of this paper is to present the mechanism of the oxido-reductive stress associated with long-term alcohol abuse. The knowledge and understanding of the mechanisms underlying this phenomenon may help to avoid the negative effects of alcohol consumption, before development of severe liver damage, induced by toxic effects of alcohol.

S t r e s z c z e n i e

Ze względu na łatwą dostępność i relatywnie niską cenę, alkohol jest obecnie najpowszechniej stosowanym środkiem odurzającym. Spożywanie nadmiernych ilości alkoholu wiąże się z wieloma konsekwencjami zdrowotnymi, do których należy powstawanie w wątrobie patologicznych zmian prowadzących do rozwoju alkoholowej choroby wątroby. Za powstawanie tych zmian odpowiedzialne są m.in. zaburzenia metaboliczne, reakcje immunologiczne i powstawanie cytokin, a także hipoksja hepatocytów okołożylnej strefy płacikowej oraz stres oksydoredukyjny. Poniższa praca ma na celu omówienie mechanizmu powstawania w organizmie stresu oksydo-redukcyjnego związanego z długotrwałym nadużywaniem alkoholu. Poznanie i zrozumienie mechanizmów leżących u podstaw tego zjawiska może pozwolić na przeciwdziałanie negatywnym skutkom konsumpcji alkoholu zanim pojawią się poważne uszkodzenia wątroby wywołane jego toksycznym oddziaływaniem.

Key words: alcohol, oxido-reductive stress, reactive oxygen species (ROS), alcoholic liver disease (ALD)
Słowa kluczowe: alkohol, stres oksydoredukcyjny, reaktywne formy tlenu (RFT), alkoholowa choroba wątroby

INTRODUCTION

Due to availability and relatively low price, alcohol is currently the most widely used intoxicant. World Health Organization reports that excessive and prolonged alcohol consumption is associated with more than 60 different diseases [1]. It was shown that there is a linear correlation between alcohol intake and development of the liver disease. Alcohol at a dose exceeding 80 g per day results in damage of liver cells [2]. Chronic alcohol abuse leads to alcoholic liver disease (ALD).

Liver is an organ which performs many important functions including metabolizing and neutralizing of toxic compounds entering the body. Alcohol is one of harmful substances being metabolized in the liver into

the final products in order to easier elimination from the body. For this reason, liver is especially vulnerable to the harmful effects of alcohol [3]. Some by-products, which are generated during these processes, are more toxic than alcohol and can damage the organ as well as contribute to the development of various diseases, including ALD. Responsible for the formation of lesions in the liver are among others: metabolic disorders, immune reactions and formation of cytokines, hypoxia of hepatocytes in the perivenular area of hepatic lobule and oxido-reductive stress [4]. The aim of the presented paper is to discuss one of the mechanisms underlying the development of alcoholic liver disease - formation of oxido-reductive stress in the body which derives from the consumption of excessive amounts of alcohol, with great emphasis on alcohol metabolism and formation of highly reactive oxygen species during these processes, which interfere oxido-reductive balance of hepatocytes.

METABOLISM OF ALCOHOL IN THE BODY

The small amount of alcohol is excreted unchanged through the kidneys and lungs. The majority of ethyl alcohol is metabolized in the liver in the presence of oxygen. For this reason, liver is an organ which is extremely vulnerable to the harmful effects of ethanol and its metabolites. The process of ethanol oxidation in the liver involves three enzymatic pathways: activity of alcohol dehydrogenase system (ADH), MEOS system (microsomal ethanol oxidizing system), and catalase [3, 5].

Reactions of oxidation involve the transfer of electrons from nicotinamide adenine dinucleotide (NAD^+), which is reduced to the form of NADH by accepting two electrons. The subsequent stages of electrons transfer to the oxygen molecule involve number of intermediate conveyors, located in the mitochondrial respiratory chain. Finally, oxygen is reduced to water in the presence of cytochrome oxidase. Cellular respiration is a highly catabolic process, during which significant amounts of energy is released [6].

During moderate consumption of alcohol, a large part of it, absorbed in the gastrointestinal tract, is oxidized. It is accompanied by alcohol dehydrogenase (ADH), which is found in many human organs, but shows the highest activity in the liver and stomach. ADH metabolizes ethanol with the participation of nicotinamide adenine dinucleotide (NAD^+). This reaction involves the isolation of hydrogen atom from the ethanol molecule and formation of acetaldehyde, while oxidized form (NAD^+) is converted into the reduced form (NADH). NADH and acetaldehyde, which are produced as a result of ethanol oxidation, are considered to be more hepatotoxic than ethanol [7].

The second enzymatic pathway is the microsomal ethanol oxidizing system (MEOS). The most important element of MEOS is multiple cytochrome P450 isoenzymes, including CYP2E1, CYP2A6, CYP1A2. Isozymes, present in microsomes or endoplasmic reticulum vesicles, are induced by chronic alcohol consumption and this is why the activity of the whole system increases with prolonged exposure to ethanol [8, 9]. Cytochrome P450 isoenzymes play an important role in the metabolism of ethanol, when its concentration is high; as it has been proven that MEOS activity is maximal when the alcohol concentration in the blood is high. Simultaneously, when the alcohol concentration in the blood is low, the oxidation process is catalyzed primarily by the enzyme ADH [7]. Although the microsomal ethanol oxidizing system is responsible for the metabolism of 10-15% of alcohol, it should be considered as an important element associated with the ethanol conversions and its harmful effects on the body, due to the fact that it contributes to generation of significant quantities of reactive oxygen species (ROS), and therefore, the formation of oxidative stress [6].

The third enzymatic pathway is observed with the participation of the catalase – an enzyme which has the ability to metabolize alcohol in the presence of hydrogen peroxide. The highest amounts of catalase is found in liver, kidney, erythrocytes, and the central nervous system, while in hepatocytes the catalase is located in peroxysomes and microsomes. The importance of this pathway in the liver is low, because catalase metabolizes less than 10% of ethanol entering this organ [10]. However, prolonged exposure to ethanol contributes to the increase of hydrogen peroxide production and thereby increased catalase activity [7]. Moreover, catalase may play a significant role in ethanol metabolism in condition of alcohol dehydrogenase deficiency [11]. It has been proven that in conditions of ADH deficiency and simultaneous 300mg of ethanol/ 100ml of blood concentration at, catalase is responsible for almost 100% of alcohol conversion, while at lower concentrations of ethanol in blood, it metabolizes approximately 50% of alcohol [12].

Acetaldehyde - the final product in all the above-mentioned enzymatic pathways - is oxidized to acetic acid under the action of aldehyde dehydrogenase (ALDH) or oxidases (xanthine or aldehyde). This reaction occurs in the mitochondria, where in addition to acetate, the reduced form of nicotinamide adenine dinucleotide (NADH) is formed. NADH is oxidized in a series of subsequent chemical transformations, e.g. in the respiratory chain [3, 7, 13].

GENERATION OF REACTIVE OXYGEN SPECIES

Due to the high chemical activity of oxygen, process of cellular respiration may be a factor that initiates the formation of the harmful reactive oxygen species. The basis of aerobic cellular respiration is the reduction of the oxygen, which reacts with organic compounds. Oxygen molecule may be fully reduced to water molecule accompanied by the energy production, and may be incompletely reduced, what leads to formation of reactive oxygen species. The largest group among ROS is formed by the free radicals - atoms, molecules or fragments of molecules charged or neutral that have one or more unpaired electrons on their outer orbital. They seek to connect with the orbitals of other atoms to form a stable chemical bond. Due to the unstable molecular structure, free radicals are forms which quickly react, in a haphazard manner. The high toxicity of free radicals is dangerous for the body, both at the cellular and molecular level. Free radicals react with lipids, proteins, nucleic acids and carbohydrates in cells, what leads to creating another free radical products that destroy or damage the cell structures [14, 15]. Free radicals of fatty acids cause the damage of cell membranes of hepatocytes. Moreover, products of lipid peroxidation play an important role in the pathogenesis of liver cell necrosis, as well as inflammation and fibrosis of the organ [16, 17].

SOURCE OF REACTIVE OXYGEN SPECIES AND FORMATION OF OXIDO- REDUCTIVE STRESS

The greatest amount of ROS in the body is a result of mitochondrial respiration process, during which about 5% of the electrons is being incompletely reduced generating free radicals. During these transformation, partially reduced form of oxygen - superoxide anion is produced, and after acceptance of the second electron, the hydrogen peroxide is formed, and the third one - the hydroxyl radical which is the most reactive compound [6, 18]. Hydrogen peroxide and superoxide anion are also produced in the enzymatic reactions catalyzed by oxidases (e.g. xanthine and NADPH) which are responsible for transferring electrons or hydrogen molecules into the atoms of oxygen [19]. Moreover, the oxidation of the respiratory proteins is a source of ROS. These are the single-electron oxidation reactions, involving the oxidation of heme groups containing the iron, with the generation of superoxide amino radical. The hydroxyl radical is formed in the free-radical reactions, catalyzed by copper and iron ions, i.e. in Fenton and Haber-Weiss reactions [20, 21]. Peroxides are formed as a result of the arachidonic acid oxidation, under the influence of enzymes responsible for transferring oxygen to the fatty acid chain (cyclooxygenase and lipoxygenase) [22].

Free radicals are formed not only through the natural metabolic processes in the body, but also as products of detoxification of many harmful substances, including alcohol. Its adverse effect is related with e.g. disturbance of the oxido-reductive balance. Production of the excessive amounts of NADH during conversion of ethanol to acetaldehyde leads to the simultaneous activation of the xanthine oxidase, what contributes to the generation of the reactive oxygen species [3, 23] . The next stage is the transformation of acetaldehyde with the participation of xanthine and aldehyde oxidases, what also leads to generation of reactive oxygen species. Overproduction of NADH during the conversion of acetaldehyde to acetic acid, as well as during the transformation of ethanol to acetaldehyde, leads to generation of oxido-reductive stress in the liver cells [16]. Metabolic consequences of this overproduction are e.g. inhibition of oxidation processes in mitochondria and increased synthesis of triglycerides and lactates in the liver [4, 15].

Certain amounts of ROS are produced in the liver with participation of the cytochrome P450 isoenzymes, including CYP2E1, which plays a special role. As already mentioned, the activity of this enzyme increases as a result of chronic alcohol abuse, and is therefore of particular importance in the study of oxidative stress induced by alcohol abuse. The activation of cytochrome P-450 is linked with generation of superoxide anion, hydroxyl radical and hydrogen peroxide, as a result of incomplete reduction of oxygen molecules [24, 25]. Moreover, during the oxidation of ethanol by the enzyme CYP2E1, the

unstable intermediate gem-diol is formed, which then decomposes to acetaldehyde. This reaction leads to oxidation of NADPH with the formation of hydrogen peroxide. The consequence of hydrogen peroxide production is the initiation of unsaturated fatty acids oxidation. Lipid peroxidation, in turn, leads to lipid peroxides level increase, as a result of large amounts of ROS. In the pathogenesis of alcohol-induced liver injury, the high levels of lipid peroxides and products of their metabolism are observed [7, 26].

At the balanced state, the proportions between production and degradation of free radicals are preserved. Overproduction of the reactive oxygen species, in the consequence of alcohol metabolism, upsets the balance and thus leads to the emergence of the oxidative stress phenomenon [15]. Excessive amounts of ROS, which are generated in case of the long-term alcohol consumption, are responsible for the lower performance of antioxidant defense mechanisms.

Disturbance of the oxido-reductive balance is also caused by a reduction in the level of the low-molecular antioxidant compounds, e.g. the glutathione and tocopherol in the liver, change in the activity of antioxidant enzymes, activation of phospholipase A_2, increasing the release of arachidonic acid, which stimulates the production of reactive oxygen species and increased absorption of iron, which catalyzes reactions of superoxide radical formation [27].

ALCOHOLIC LIVER DISEASE

Chronic consumption of alcohol contributes to formation of the oxidative stress in the body, what is associated with the metabolism of ethanol. As a result of oxidation of ethanol, the significant amount of free radicals is produced, a change in the level of NADH and the ratio of NADH/ NAD^+ occurs, what consequently leads to the oxidative stress in the liver. The reduction of oxidized and reduced forms proportion leads to disturbance of oxydo-reductive balance in hepatocytes [7]. Development of alcoholic liver disease (ALD) is the consequence of prolonged and chronic alcohol consumption. ALD is characterized by varying degrees of organ damage: steatosis, hepatitis, fibrosis and cirrhosis [26]. In case of individuals who consume alcohol in large quantities, steatosis may progress to hepatitis, then cirrhosis. Signs of all three phases may occur in some patients at the same time, but in about 20% of alcoholics and alcohol abusers develop fatty liver disease, and in many cases, in addition to hepatomegaly (enlarged liver), no other clinical symptoms occur [26]. Steatosis occurs as a result of oxidative imbalance between production and oxidation of free fatty acids in hepatocytes. Then, liver is attacked by free radicals, which arise as a result of the activity of MEOS, in excessive amounts. This state could be reversed by introducing the abstinence or limitation of alcohol intake [16]. At the same time this state can be fatal as long as the patient will not completely resign from drinking alcohol or will not limit its consumption. The next stage of alcoholic liver disease – heaptitis is diagnosed when the biopsy reveals the inflammation, degeneration, fibrosis or other abnormalities in hepatocytes. Clinical manifestations of hepatitis include: abdominal pain, nausea, vomiting and liver swelling. Moreover, patients may suffer from: fever, jaundice and liver failure. In about 40% of cases, if alcohol consumption is continued, inflammation of the liver converts to cirrhosis, which is the most severe form of alcoholic liver disease [26]. At this stage, the fibrosis occurs, which is caused by replacement of liver parenchyma cells by the connective tissue fibers, which impairs blood flow through the organ and disrupts its proper functioning. The activators of Kupffer cells, which induce organ fibrosis, are the products of ethanol metabolism [28]. Clinical signs, besides the red hand, fibrosis of tendons in the hands, fingers and nails deformation, are also enlargement of the liver, inflammation and abnormal accumulation of fat in hepatocytes, what causes scarring of the liver. Cirrhosis is diagnosed based on biopsy and additional laboratory studies [26]. This is an irreversible state and its complications include encephalopathy and renal failure. Cirrhosis is also considered as the one of the risk factors for liver cancer [16].

CONCLUSIONS

The harmful effect of alcohol on the organism is the result of wide range of factors and its toxic influence on liver depends on e.g. the genetically determined activity of enzymes metabolizing ethanol determining the individual alcohol sensitivity , overall health condition, alcohol intake and exposure time [4, 28, 29]. Responsible for the formation of lesions in the liver are among others: metabolic disorders, immune reactions and formation of cytokines, hypoxia of hepatocytes in the perivenular area of hepatic lobule and oxido-reductive stress [4]. Knowing and

understanding the mechanisms underlying phenomenon of oxidative stress in the body, related to the production of reactive oxygen species by metabolism of excessive amounts of alcohol may help to avoid the negative effects of alcohol consumption, before developing severe liver damage caused by its toxic effects.

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Address for correspondence:

Aleksandra Kołota
Zakład Fizjologii Żywienia, Katedra Dietetyki,
Wydział Nauk o Żywieniu Człowieka
i Konsumpcji
Szkoła Główna Gospodarstwa Wiejskiego
w Warszawie
02-766 Warszawa
ul. Nowoursynowska 159c
tel. (22) 593 70 29
fax (022) 593 70 31
e-mail: aleksandra_kolota@sggw.pl

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